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MERCHANT & GOULD PC
P.O. BOX 2903
MINNEAPOLIS, MN 55402-0903

EXAMINER	
LONG, SCOTT	
ART UNIT	PAPER NUMBER
1633	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/11/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/685,693	WEST ET AL.	
	Examiner	Art Unit	
	Scott D. Long	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 November 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-157* is/are pending in the application.

4a) Of the above claim(s) *27-32,35-59,78,79,81-88,91,93 and 103-157* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-26, 33-34, 60-77, 80, 89-90,92, 94-102* is/are rejected.

7)☒ Claim(s) *16, 67, 70* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *16 October 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Examiner acknowledges the election, with traverse, of Group I (claims 1-102) directed to *ex vivo* methods of identifying genes and determining relative timing of transcriptional activation or repression of said genes in stem cells during differentiation, in the reply filed on 20 November 2006. The examiner also acknowledges the election, without traverse, of Species Ia, a nucleotide sequence that encodes a protein, specifically a fluorescent protein, which read on claims 1-14, 16-80, 83-90, 92, and 94-102. The examiner also acknowledges the election, with traverse, of Species I-5, a human embryonic stem cell, which read on claims 1-42, 60-83, and 89-102.

Because no argument for the traversal was provided by applicant, thus the traversal is non-persuasive and the restriction is made final.

Claim Status

Claims 1-157 are pending. However, claims 27-32, 35-59, 78-79, 81-88, 91-93 and 103-157 are withdrawn from further consideration by the Examiner, pursuant to 37 CFR 1.142(b), as being drawn to non-elected inventions, there being no allowable generic or linking claim. Claims 1-26, 33-34, 60-77, 80, 89-90, and 94-102 are under current examination and will be examined to the extent to which they read on *ex vivo* methods and the species elections cited above.

Oath/Declaration

The oath or declaration, having the signatures of all inventors, received on 28 June 2004 is in compliance with 37 CFR 1.63.

Information Disclosure Statement

The Information Disclosure Statements (IDS) filed on 12 January 2005 and 10 June 2005 consisting of 3 sheets are in compliance with 37 CFR 1.97. Accordingly, examiner has considered the Information Disclosure Statements.

Priority

This application claims benefit from provisional U.S. Application No. 60/418,333, filed 16 October 2002. The instant application has been granted the benefit date, 16 October 2002, from the application 60/418,333.

Claim Objections

Claim 16 is objected to because of the following informalities: There is a typo in claim 16, "screeding". Appropriate correction is required.

Claim 67 is objected to because of the following informalities: There is a duplication of the word, "genes", in line 5. Appropriate correction is required.

Claim 70 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is

required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. There seems to be no difference between Claim 67, "...two or more different endogenous genes, in each of which is inserted a marker DNA construct" and claim 70, "cells contain more than one marker gene".

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-17 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. There is a disconnect between the preamble, "determining the relative timing of transcriptional activation" and the outcome of the method, "detecting differentiating cells." If the insertion of the reporter gene were random, then many insertions would not change expression levels. If the insertion of the reporter gene is random, then some type of characterization of the stem cell line would need to be performed, so that the random "change of expression levels" could be associated with a particular cell lineage. The omitted steps are: either characterizing insertion site or characterizing specific cell lineage that is affected by gene trap construct.

Claim 64 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 64 is indefinite in that there is a blank at the end of the claim.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-8, 16-26, 60-69, 71, 90, 94-96 are rejected under 35 U.S.C. 102(b) as being anticipated by Stanford et al. (Blood, Vol 92, No 12 (December 15), 1998: pp 4622-4631).

The instant invention is directed to a method for determining the relative timing of the transcriptional activation or repression of genes in a population of stem cells that occurs when the stem cells differentiate, comprising: (a) randomly inserting into the genomic DNA of stem cells in a population of stem cells a marker DNA construct comprising a nucleotide sequence that encodes a detectable product and is not operably linked to a promoter; (b) culturing the stem cells ex vivo under conditions in

which the stem cells differentiate; (c) monitoring the differentiating stem cells to detect changes in the level of expression of the marker DNA constructs in the cells; and (d) detecting differentiating cells in which there is a change in the level of expression of the marker DNA construct, and determining the relative timing of the change in the level of expression of the marker DNA construct that occurs in these cells.

Stanford et al. teach "random insertion of exogenous DNA into single sites in the mammalian genome" (page 4622, column 2). Stanford et al. apply this method to identify and characterize a large number of genes capable of lineage-specific expression in murine embryonic stem cells during differentiation (page 4622, column 2). The system uses a promoterless *lacZ* reporter gene that catalyzes a chromogenic product, when integrated downstream of an endogenous promoter (page 4623). The ES cells were grown for a series of days (page 4624, column 1) until the "transcriptional activation of the trapped gene" (page 4624, column 2) and subsequent *lacZ* expression (blue staining) during *ex vivo* differentiation (page 4624). Stanford et al. teach, "pattern and levels of *lacZ* expression" (page 4626-4627) at various stages of differentiation of ES cells into hematopoietic and mesodermal lineages. Stanford et al. teach differentiation of isolated clones of gene trapped ES cells (page 4628, figure 4). The method of Stanford et al. further comprises sequence analysis of gene where integration occurs (page 4622, abstract). Stanford et al. teach totipotent embryonic stem cell lines (page 4622, line 2). Stanford et al. teach their method identifies multiple genes (page 4622). The method of Stanford further utilizes Southern Blotting and hybridization (page 4624, column 1). Stanford et al. identified two or more different

genes which are transcriptionally activated at different times during differentiation into a particular cell type (page 4625, table 3). Stanford et al. assayed to detect a change in expression of LacZ polypeptide (page 4624). Stanford et al. teach induction of stem cells to progenitor cells of hematopoietic and vascular cell lineages and further differentiation into fully differentiated "circulating blood cells" (page 4626, column 2). Stanford et al. teach the further development of the ES cells into embryoid bodies (EB) (page 4624, Results). The teachings of Stanford et al. include use of RACE PCR to determine mRNA expression. Stanford et al. also utilize RACE fragment probes for hybridization.

Accordingly, Stanford et al. anticipated the instant claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation

under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-8, 10, 16-26, 60-76, 90, 92, 94-100 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stanford et al. (Blood, Vol 92, No 12 (December 15), 1998: pp 4622-4631) in view of Nehls et al (US Patent 6,218,123, issued April 17, 2001).

The instant invention is directed to a method for determining the relative timing of the transcriptional activation or repression of genes in a population of stem cells that occurs when the stem cells differentiate, comprising: (a) randomly inserting into the genomic DNA of stem cells in a population of stem cells a marker DNA construct comprising a nucleotide sequence that encodes a detectable product and is not operably linked to a promoter; (b) culturing the stem cells ex vivo under conditions in which the stem cells differentiate; (c) monitoring the differentiating stem cells to detect changes in the level of expression of the marker DNA constructs in the cells; and (d) detecting differentiating cells in which there is a change in the level of expression of the marker DNA construct, and determining the relative timing of the change in the level of expression of the marker DNA construct that occurs in these cells.

The teachings of Stanford et al. are described above in the 35 USC 102(b) section.

Stanford does not teach the use of human embryonic stems cells in their method, but does satisfy the limitation of some species of claim 4, particularly murine ES cells.

Also, Stanford et al. do not teach the use of fluorescent proteins in their gene trap construct, but rather utilize the colorimetric catalyzing *lacZ* gene, which is one of the unelected species listed in claim 5. Stanford et al. does not explicitly teach a method that employs more than one marker gene within the same cell, but clearly teach the use of a single marker within multiple cells.

Nehls et al. teach methods of screening gene trap cassettes integrated into the genome comprising selectable markers, including chromogenic and fluorescent markers (column 4, lines 20-25). The "gene trap selection is employed...[on]...preferred target cells include...embryonic stem cells, and particularly human embryonic or other stem cells" (column 4, lines 40-43). Nehls et al. teach, "selectable marker may be expressed by control elements present in the vector, or, preferably, the selectable marker is only expressed under the control of an endogenous, i.e. cellular, promoter. This feature allows one to select for both the integration event, and also better insures that the construct has integrated within a cellular gene." (column 4, lines 25-31).

In particular, Nehls et al. teach a method of gene trapping employing multiple selectable markers (column 3, line 41) and multiple genes (Figure 4) expressed by endogenous promoters (column 4, line 28).

Nehls et al do not specifically teach changes in levels of expression, because of their invention utilizes a normalized library isolated from stem cells.

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to utilize a fluorescent protein in the gene trap system of Stanford et al. It would have been obvious to the person of ordinary skill in the art at the time the

invention was made to apply the gene trap system of Stanford et al. to human embryonic stem cells.

The person of ordinary skill in the art would have been motivated to make those modifications because fluorescent proteins, such as Green Fluorescent Protein (GFP) are functionally equivalent to lacZ (β-galactosidase) systems and do not require further reagents, such as X-gal, for visualization, as is the case for β-galactosidase. In addition, Stanford et al. teach that gene trap analysis of human embryonic stem cell gene expression is important for "understanding normal physiological processes and human disease" (page 4622, column 1).

The skilled artisan would have had a reasonable expectation of success in combining the teachings of Stanford et al. and Nehls et al. because each of these teachings generated successful use of the gene trap technology and Stanford in particular, utilized their method to identify genes expressed at different times during the course of differentiation of from ES cells into various cell lineages.

Therefore the method as taught by Stanford et al. in view of Nehls et al. would have been *prima facie* obvious over the method of the instant application.

Claims 1-10, 16-26, 60-69, 71, 77, 80, 90, 94-96, and 101-102 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stanford et al. (Blood, Vol 92, No 12 (December 15), 1998: pp 4622-4631) in view of Stanford et al (Nature. October 2001. Vol.2, pp. 756-768).

The instant invention (claims 101-102) are directed to a method for determining the relative timing of the transcriptional activation or repression of genes in a population of stem cells that occurs when the stem cells differentiate, comprising: (a) randomly inserting into the genomic DNA of stem cells in a population of stem cells a marker DNA construct comprising a nucleotide sequence that encodes a detectable product and is not operably linked to a promoter; (b) culturing the stem cells ex vivo under conditions in which the stem cells differentiate; (c) monitoring the differentiating stem cells to detect changes in the level of expression of the marker DNA constructs in the cells; and (d) detecting differentiating cells in which there is a change in the level of expression of the marker DNA construct, and determining the relative timing of the change in the level of expression of the marker DNA construct that occurs in these cells, wherein the marker DNA construct encodes a recombinase, wherein the same or a different marker DNA construct inserted into the genomic DNA of each stem cell comprises a first nucleotide sequence encoding a detectable product, and a second nucleotide sequence comprising two recombination sites that inhibits the expression of the first nucleotide sequence; wherein the recombinase recognizes the two recombination sites, and expression of the nucleotide sequence encoding recombinase results in excision of the second nucleotide sequence from the stem cell genomic DNA and expression of the first nucleotide sequence encoding the detectable product; and wherein step (d) comprises determining the relative timing of the transcriptional activation of the marker DNA constructs that occurs when the stem cells differentiate.

Claim 77 is directed to the further limitation of claim 71, wherein one marker is recombinant mediated and the other marker is randomly inserted.

The teachings of Stanford et al. (Blood, Vol 92, No 12. 1998: pp 4622-4631) are described above in the 35 USC 102(b) section, satisfying the limitations of claims 1, 67, 71. Stanford et al. also teach the limitation of claim 80, assays comprising radiolabeled hybridization probes, "RACE fragment probes" (page 4624, column 1).

Stanford et al. (1998) do not teach the further limitation of claim 101-102, wherein expression of a recombinase gene permits expression of a detectable product through use of two recombination sites. Stanford et al. only teach a portion of the limitation of claim 77, random insertion of marker gene construct.

Stanford et al. (Nature. October 2001. Vol.2, pp. 756-768) teach a method to "increase the versatility of trapping, several groups have modified vectors to include recombination sites,...permits additional modifications to be made to a trapped locus, such as co-opting the promoter elements of the trapped gene to drive the expression of a knocked-in transgene for use in...cell-labelling experiments" (page 763, column 1). The teachings of Stanford et al (October 2001) meet the further limitations of claim 101, requiring integration of DNA comprising recombinase gene and recombination sites and gene expressing detectable product. In particular, Stanford et al. (2001) teach the limitations of claim 77, specifically "homologous recombination in embryonic stem (ES) cells" (page 759, column 2) and "random mutagenesis" (page 759, column 2).

The person of ordinary skill in the art would have been motivated to make those modifications because "by combining homologous recombination and gene-trapping

strategies, gene trapping can be used for more than just expression, sequence and simple functional analysis" (Stanford et al. 1998), such as post-insertional modifications of the gene-trap locus.

In addition, Stanford et al. teach that gene trap analysis of human embryonic stem cell gene expression is important for "understanding normal physiological processes and human disease" (page 4622, column 1).

The skilled artisan would have had a reasonable expectation of success in combining the teachings of Stanford et al. (1998) and Stanford et al. (2001) because each of these teachings generated successful use of the gene trap technology and Stanford (1998) in particular, utilized their method to identify genes expressed at different times during the course of differentiation of from ES cells into various cell lineages.

Therefore the method as taught by Stanford et al. in view of Stanford et al. would have been *prima facie* obvious over the method of the instant application.

Claim 89 is rejected under 35 U.S.C. 103(a) as being unpatentable over Stanford et al. (Blood, Vol 92, No 12 (December 15), 1998: pp 4622-4631) in view of Stanford et al (Nature. October 2001. Vol.2, pp. 756-768) as applied to claims 67, 71, and 77 above, and further in view of Odorico et al (STEM CELLS. 2001;19:193-204).

The teachings of both Stanford et al. references are detailed above in the previous 35 USC 103(a) section.

The Stanford et al. references do not teach the injection of gene-trapped stem cells into non-human mammals to form teratomas, and further detection of marker transcription.

Odorico et al. teach, "in vitro differentiation of ES cells transduced with gene trap vectors can be used to discover novel developmentally regulated genes that are important in tissue specific differentiation programs" (page 196, column 1). Odorico et al. further teach, "Human ES cells injected into severe combined immunodeficient mice form benign teratomas, with advanced differentiated tissue types representing all three EG layers" (page 197, column 2).

The person of ordinary skill in the art would have been motivated to make those modifications because "Embryonic inductive events and complex epithelial-mesenchymal interactions control the formation of organized tissue structures during normal embryogenesis. These events and interactions begin to occur in teratomas but are less pronounced during in vitro differentiation." (Odorico et al., page 197, col. 2).

The skilled artisan would have had a reasonable expectation of success in combining the teachings of Stanford et al. (1998) and Stanford et al. (2001) with Odorico et al. because each of these teachings generated successful use of the gene trap technology and Stanford (1998) in particular, utilized their method to identify genes expressed at different times during the course of differentiation of from ES cells into various cell lineages.

Therefore the method as taught by Stanford et al. in view of Stanford et al. and further in view of Odorico et al. would have been *prima facie* obvious over the method of the instant application.

Claims 1-2, 11-14, 18, and 33-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stanford et al. (Blood, Vol 92, No 12 (December 15), 1998: pp 4622-4631) in view of Chajut et al (WO/2002/086089, filed, April 23, 2002).

The instant invention is directed to a method for determining the relative timing of the transcriptional activation or repression of genes in a population of stem cells that occurs when the stem cells differentiate, comprising: (a) randomly inserting into the genomic DNA of stem cells in a population of stem cells a marker DNA construct comprising a nucleotide sequence that encodes a detectable product and is not operably linked to a promoter; (b) culturing the stem cells ex vivo under conditions in which the stem cells differentiate; (c) monitoring the differentiating stem cells to detect changes in the level of expression of the marker DNA constructs in the cells; and (d) detecting differentiating cells in which there is a change in the level of expression of the marker DNA construct, and determining the relative timing of the change in the level of expression of the marker DNA construct that occurs in these cells, step further comprising isolating individual stem cells having an inserted marker DNA construct, or colonies of cloned cells derived from such individual stem cells; and then culturing the isolated stem cells in step (b), further comprising purifying differentiating cells having a

transcriptionally activated marker DNA construct from cells that do not have a transcriptionally activated marker DNA construct, and using the purified cells or an extract thereof as an immunogen to elicit production of an antibody that binds specifically to a differentiation antigen of the purified cells.

The teachings of Stanford et al. are described above in the 35 USC 102(b) section, which satisfy the limitation of claims 1-2 and claim 18, regarding the general use of the gene trap system to identify and quantify differentiation-specific genes in embryonic stem cells.

Stanford does not teach a method generating differentiation stage antibodies from purified cells or cell extracts.

Chajut et al. teach "present invention relates to the identification of genes involved in proliferation and differentiation of embryonic stem cells" (page 1, lines 11-12). Chajut et al. teach, "present invention provides for use of said differentiation factor for determination of the differentiation stage of selected cells." (page 12, lines 4-5). Chajut et al. further teach, "antibodies may be prepared against the immunogen or antigenic portion thereof, for example,...the natural gene product and/or portions thereof" (page 19, Antibody production) and further describe immunization of non-human mammals to produce antibodies (page 20).

Chajut et al. does not teach methods of identifying genes using the gene trap system.

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to apply the gene trap system of Stanford et al. to stem cells and to further generate antibodies to the differentiation specific genes identified by the method.

The person of ordinary skill in the art would have been motivated to make those modifications because "antibodies may be used in various aspects of the invention, e.g., in detection or treatment or prevention methods. Antibodies can be monoclonal, polyclonal or recombinant for use in the immunoassays or other methods of analysis" (Chajut et al. page 19, lines 20-22).

The skilled artisan would have had a reasonable expectation of success in combining the teachings of Stanford et al. and Chajut et al. because each of these teachings generated successful use of the gene trap technology and Stanford in particular, utilized their method to identify genes expressed at different times during the course of differentiation of from ES cells into various cell lineages, while Chajut et al. further utilizes cell extracts to generate antibodies specific to differentiation-specific antigens.

Therefore the method as taught by Stanford et al. in view of Chajut et al. would have been *prima facie* obvious over the method of the instant application.

Conclusion

No claims are allowed.

Examiner Contact Information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Scott Long** whose telephone number is **571-272-9048**. The examiner can normally be reached on Monday - Friday, 9am - 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Joseph Woitach** can be reached on **571-272-0739**. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Scott Long
Patent Examiner
Art Unit 1633

JANET L. EPPS-FORD, PH.D.
PRIMARY EXAMINER